Institute Report No. 283

Mutagenic Potential of Nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test

Raj K. Gupta, PhD, MAJ, MSC
Vilmar O. M. Villa, BS, SPC
and
Don W. Korte, Jr., PhD, MAJ, MSC

GENETIC TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

August 1988

Toxicology Series: 209

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Mutagenic Potential of Nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test (Toxicology Series 209)--Gupta *et al.*

This document has been approved for public release and sale; its distribution is unlimited.

Destroy this report when it is no longer needed. Do not return to the originator.

Citation of trade names in this report does not constitute an official endorsement or approval of the use of such items.

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. *(AR 360-5)*

Edwin S. Beatrice 5 Aug 88

Edwin S. Beatrice (date)
COL, MC
Commanding

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 283	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Division of Toxicology; 6b. OFFICE SYMBOL (If applicable): SGRD-ULE-T	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62720A	835	AB	DA 303913

11. TITLE (Include Security Classification): Mutagenic potential of nitrosoguanidine in the Drosophila melanogaster sex-linked recessive lethal test

12. PERSONAL AUTHOR(S): Raj K. Gupta, Vilmar O. M. Villa, and Don W. Korte, Jr.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
final	FROM _____ TO _____	August 1988	17

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	mutagenicity, genetic toxicology, Sex-Linked Recessive Lethal Assay, Drosophila melanogaster, nitroguanidine

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Nitrosoguanidine, an anticipated degradation product of nitroguanidine which in turn is a primary component of U.S. Army triple-base propellants, was evaluated for mutagenic potential in the Drosophila melanogaster Sex-Linked Recessive Lethal test. Nitrosoguanidine was non-mutagenic following 72-hour feeding exposures to concentrations of nitrosoguanidine ranging from 5 mg/ml to 50 mg/ml.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Edwin S. Beatrice, COL MC	415-561-3600	SGRD-ULZ

DD Form 1473, JUN 86 — Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

ABSTRACT

Nitrosoguanidine, an anticipated degradation product of nitroguanidine which in turn is a primary component of U.S. Army triple-base propellants, was evaluated for mutagenic potential in the *Drosophila melanogaster* Sex-Linked Recessive Lethal test. Nitrosoguanidine was non-mutagenic following 72-hour feeding exposures to concentrations of nitrosoguanidine ranging from 5 mg/ml to 50 mg/ml.

Key Words: Mutagenicity, Genetic Toxicology, Sex-Linked Recessive Lethal Assay, *Drosophila melanogaster*, Nitroguanidine.

PREFACE

TYPE REPORT: *Drosophila melanogaster* Sex-Linked Recessive Lethal Test

TESTING FACILITY: U.S. Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: U.S. Army Medical Research and Development Command
U.S. Army Biomedical Research and Development Laboratory
Fort Detrick, Frederick, MD 21701-5010

PROJECT/WORK UNIT/APC: 3E16270A835/180/TLBO

GLP STUDY NUMBER: 86001

STUDY DIRECTOR: MAJ Don W. Korte, Jr., PhD, MSC

PRINCIPAL INVESTIGATOR: MAJ Raj K. Gupta, PhD, MSC

CO-PRINCIPAL INVESTIGATOR: SPC Vilmar O. M. Villa, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, test compound sample, raw data, and appropriate SOPs will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitrosoguanidine

INCLUSIVE STUDY DATES: 1 May 1986 - 7 August 1987

OBJECTIVE: The objective of this study was to evaluate the mutagenic potential of nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test.

ACKNOWLEDGMENT

The investigators wish to thank Dr. Stanley T. Omaye for critical review of this report.

Signatures of Principal Scientists Involved in the Study

We, the undersigned, declare that the GLP Study 86001 was performed under our supervision, according to the procedures described herein, and that the report is an accurate record of the results obtained.

Don W. Korte Jr. 2 August 88
DON W. KORTE JR., PhD/Date
MAJ, MSC
Study Director

Rajkumar Gupta 2 Aug '88
RAJ K. GUPTA, PhD/Date
MAJ, MSC
Principal Investigator

for Rajkumar Gupta 2 Aug '88
VILMAR O. M. VILLA, BS/Date
SP4, USA
Biological Sciences Assistant

Conrad Wheeler 2 Aug 1988
CONRAD WHEELER, PhD/Date
DAC
Analytical Chemist

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF

SGRD-ULZ-QA (70-1n) 2 August 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 86001

1. This is to certify that in relation to LAIR GLP Study 86001, the following inspections were made:

09 March 1987	-	Media Preparation
18 March 1987	-	CS Exposure
25 March 1987	-	Brood 3 Mating
06 April 1987	-	F_1 Matings
22 April 1987	-	Score F_2 Generation, Brood 1
11 June 1987	-	CS Exposure, Males, Run 65
12 June 1987	-	F_1 Mating, Brood 1
29 June 1987	-	F_1 Mating, Brood 1
20 July 1987	-	F_2 Scoring, Brood 2, Run 65

2. The institute report entitled "Mutagenic Potential of Nitrosoguanidine in the Drosophila melanogaster Sex-linked Recessive Lethal Test," Toxicology Series 209, was audited on 18 July 1988.

Walter G. Bell

WALTER G. BELL
SFC, USA
Auditor, Quality Assurance

TABLE OF CONTENTS

Mutagenic Potential of Nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test--Gupta *et al.*

INTRODUCTION

Nitrosoguanidine is a potential anaerobic degradation product of nitroguanidine (1), a primary component of U.S. Army triple-base propellants, and is now produced in a Government-owned contractor-operated ammunition plant. The U.S. Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants generated by U.S. Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (2). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine and related intermediates/by-products of its manufacture, and its environmental degradation products such as nitrosoguanidine. This study evaluated the mutagenic potential of nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal (SLRL) Test.

Rationale for SLRL Testing

A variety of tests using *Drosophila melanogaster* are available for the detection of specific types of genetic changes. The most sensitive assay that detects the broadest range of mutations is the SLRL test (3-5). This test uses insects of a known genotype and detects lethal mutagenic changes in 800 to 1000 loci on the X-chromosome, representing 80% of the X-chromosome or 20% of the entire genome (6,7). The SLRL test is used frequently to assess the mutagenic response of *Drosophila melanogaster* to test substances (3,5,6).

Genetic Basis of the SLRL Test

The genetic basis of the SLRL test is that the X-chromosome of the father is passed on to the daughter; the sons receive their X-chromosome from the mother. Therefore, the recessive lethal mutations located on the X-chromosomes are expressed in males in a hemizygous condition, i.e. the Y-chromosome does not contain the dominant, wild-type alleles to suppress the manifestation of recessive lethal mutations.

Consequently, among the progeny of a female carrying a recessive lethal mutation on one of her X-chromosomes (heterozygous for a recessive lethal mutation), half of the male progeny die. By using suitable genetic markers, the class of males carrying the mutated X-chromosomes of treated grandfathers can be determined easily. If a lethal mutation were induced, this class would be missing and its absence easily scored. The SLRL test has also been called the Basc or Muller-5 test (7,8). The assay system must use strains in which crossing-over in females is prevented since transfer of the lethal mutation from the paternal to the maternal X-chromosome by genetic recombination would suppress its expression. The crossing-over would lead to erroneous study results because males receiving that X-chromosome would survive. Since combinations of suitable inversions effectively inhibit crossing-over, females used for the SLRL test carry two scute inversions: the left-hand part of scS1 and the right-hand part of sc8 covering the whole X-chromosome and a smaller inversion In-S in the Basc chromosome (7).

Description of Test

The SLRL test (9) was developed in 1948 for determining genetic changes that kill the developing individual (egg to pre-adult stage) in the hemizygous, but not homozygous or heterozygous, conditions. Such genetic changes, i.e., recessive lethal mutations, can be induced on all chromosomes. Only two test generations are needed to detect whether sex-linked recessive lethal mutations have been induced on the X-chromosome. In the test, males with normal round red eyes (Canton-S (CS)) whose chromosomes contain wild-type alleles are exposed to nitrosoguanidine. Such an exposure will produce a recessive lethal mutation if the X-chromosome is affected. These males are mated to homozygous Basc females. The Basc phenotype is characterized by bar (narrow-shaped) eyes which are apricot in color. The bar eyes serve as a genetic marker for the homozygous and hemizygous genotypes since in the heterozygous expression the eyes are kidney-shaped. The progeny of this cross (CS males X Basc females) consists of females heterozygous for the

treated X-chromosome, characterized by kidney-shaped red eyes and males of the Basc phenotype that have received their X-chromosome from their Basc mother. Each F1 female possesses one paternal X-chromosome which was exposed to the test compound in the male gamete. F1 siblings are allowed to mate; they produce the F2 generation. The F2 generation will consist of males of two phenotypic expressions and females of two phenotypic expressions. The male phenotypes have round red eyes (hemizygous carrying the treated X-chromosome from the F1 female) or bar-shaped apricot eyes (hemizygous for the Basc chromosome). The female phenotypes carry the chromosomes for red eyes (heterozygous, carrying the treated X-chromosomes from the F1 female and the Basc chromosome) or chromosomes for bar-shaped apricot eyes (homozygous for the Basc chromosome). The F2 generation is then inspected for the presence of males with round eyes. If this class is missing, it can be concluded that the treated male gamete contained a recessive lethal mutation. Thus, this test relies upon the disappearance of a whole Mendelian class (males with round red eyes).

A brooding technique was used to sample sperm cells exposed to the test chemical during different stages of spermatogenesis because chemicals often exhibit stage specific mutagenicity. Brooding was accomplished by transferring the treated males to vials containing fresh virgin females at intervals of 1, 4, 6, and 8 days after completion of the dosing period. This technique assures that the four broods of females are inseminated with sperm exposed to the test chemical during successive stages of germ cell development: Brood 1 = mature sperm (Days 1-3); Brood 2 = primarily spermatids (Days 4-5); Brood 3 = primarily meiotic stages (Days 6-7); and Brood 4 = primarily spermatogonia (Days 8-10). This procedure safeguards against the possibility that chemicals with more pronounced effects in earlier stages of spermatogenesis are not dismissed erroneously as false negatives.

Objective of Study

The objective of this study was to evaluate the mutagenic potential of nitrosoguanidine in the *Drosophila melanogaster* Sex-Linked Recessive Lethal Test.

MATERIALS AND METHODS

Test Substance

Chemical name: Nitrosoguanidine

CAS Registry #: 674-81-7

Molecular formula: CH_4N_4O

Other test substance information is presented in Appendix A.

Vehicle

The solubility, stability, and toxicity of a number of compounds were tested to determine the compounds' suitability as a vehicle. A suspension of 1% carboxymethyl-cellulose and 1% fructose in water was found to be an appropriate vehicle for the nitrosoguanidine.

Test Model

Insect Genus and Species: *Drosophila melanogaster*

Strains: Canton-S (CS), a wild-type stock, characterized by round red eyes, was selected for mutagenicity studies because it has shown a low spontaneous mutation frequency (10).

Basc, a laboratory stock, homozygous in females, possesses bar-shaped, apricot-colored eyes and scute as phenotypic markers. The genetic designation is $In(1)sc^{S1}Lsc^{8R}IN(1)S$, sc^{8}, $sc^{S1}w^{a}B$.

Both strains are presently being reared in the insectary at Letterman Army Institute of Research. The original stock colonies were obtained from the Mid-American *Drosophila* Stock Center, Bowling Green State University, Bowling Green, Ohio.

Diet

The diet was the standard medium consisting of cornmeal (NBCO Chemicals), unsulphured molasses (Ingredient Technology Corp.), yeast (Nabisco Brands, Inc.), and nutrient agar (Moorhead & Co., Inc.) used for colony rearing of *Drosophila melanogaster*. A materials list and instructions for its preparation are contained in LAIR SOP-OP-STX-5 "Drosophila Media Preparation."

Restraint

Ether (J. T. Baker Chemical Co.) anesthesia was used to restrain flies being collected for mating and for general colony maintenance.

Identification System

Each CS male from the 72-hour LC_{50} exposure (test, negative, and positive control) was assigned a unique number. This number was also placed on the vial in which its progeny was produced (LAIR SOP-OP-STX-8 "Sex-Linked Recessive Lethal (SLRL) *Drosophila melanogaster* Mutagenicity Test"). In this manner progeny could be traced back to the parental male which had been subjected to the test compound or control vehicle.

Storage of Raw Data

Tabular data from this study (GLP 86001) for each male are in the archives of Letterman Army Institute of Research, Presidio of San Francisco, California.

Environmental Conditions

All studies were conducted within the insectary at an average temperature of 23 ± 2°C (range 21° to 26°C), relative humidity of 57 ± 8%, and a photoperiod of 12 hours light and 12 hours dark. All insect colonies were reared in polypropylene bottles, and those used in the SLRL testing were housed in glass vials (LAIR SOP-OP-STX-6 "Drosophila Stock Colony Maintenance").

Dosing

A number of preliminary studies were conducted to test potential dosages for toxicity to flies and their ability to feed and digest nitrosoguanidine in an appropriate medium. A feeding suspension vehicle of 1.0% carboxymethyl-cellulose and 1% fructose in deionized water was selected for the study. The pH of all the stock solutions including the negative control and positive controls through the study ranged from 6.7 to 8.8.

Dosing was accomplished in compliance with LAIR SOP-OP-STX-7 "*Drosophila melanogaster* Exposure Procedures." The CS strain (wild-type) males were allowed to feed on 250 µl of various concentrations of the test chemical. These males formed the test groups. Concurrent exposure to 1% carboxymethyl-cellulose and 1% fructose in deionized water was designated as the negative control group. A positive

control group was exposed to a 1.0-mM ethylmethane sulfonate solution formulated with 1% carboxymethyl-cellulose and 1% fructose in water. Ethylmethane sulfonate is a known mutagen and was used to confirm the ability of the test organism to produce SLRL mutations (11). Dosing was continuous for 72 hours. Flies were transferred every 24 hours to vials containing fresh compound suspensions. The concentrations used for the LC_{50} determinations were 5, 10, 15, 20, and 50 mg/ml nitrosoguanidine. The LC_{50} determinations were conducted 4 times, once for each replicate. The concentration for each replicate that produced a 72-hour mortality closest to 50% was selected, and only flies raised on this concentration were used in the subsequent crosses.

Test Format

The CS males surviving the LC_{50} (approximate) dose of the test chemical and those males subjected to the concurrent negative controls were used in the SLRL test. Twenty-five CS male (wild-type) survivors of the test chemical and negative control compound were scored by mating to Basc virgin females (Basc chromosomes). This procedure was accomplished by placing 3 Basc virgin females in a vial with one CS male. The vial was labeled with the male's unique number. At days 1, 4, 6, and 8 after dosing, the CS male was transferred to successive groups of 3 Basc virgin females in vials with that male's unique number. These intervals corresponded to broods 1, 2, 3, and 4. This procedure was replicated 4 times. Scoring of the mutants resulting from positive control exposure was based on mating 5 CS males in the same manner as males treated with the test compound. This procedure was also replicated 4 times. After sufficient numbers of flies had emerged, a maximum of 25 (minimum of 5) kidney-shaped red-eyed F1 females were selected at random and mated with their sibling white-body, bar-shaped, apricot-eyed males. Each pair was placed in an individual vial, and these vials from the same uniquely numbered father were placed together and labeled with his unique number for reference. After 2 to 3 weeks the F2 progeny were examined and scored for the absence of round, red-eyed males, which would indicate that a lethal mutation had taken place in the treated male. Confirmation of a lethal mutation was obtained by conducting an F3 cross from each vial scored as a lethal mutation. This was accomplished by crossing three F2 females (kidney-shaped red eyes) with three males with bar-shaped white-apricot eyes, in three separate vials (one male and one female per vial). Absence of males with round, red eyes in the resulting F3 generation confirmed the existence of a recessive lethal mutation. Experimental conclusions were based on the spontaneous mutation frequency (negative control) compared to the mutation frequency induced by the

test chemical. This entire procedure was replicated 4 times to obtain a sufficiently large sample.

Historical Listing of Significant Study Events

Appendix B is a historical listing of significant study events.

Statistical Analysis

This testing was designed to examine approximately 2500 X-chromosomes in each of 4 replications, thereby yielding a total of 8000 to 10,000 X-chromosomes for examination. Vials without F2 progeny or fewer than 5 progeny (F2) were scored as failures. The BMDP (Biomedical Programs) computer package was used to perform the analyses (12). Based on the number of lethal and nonlethal offspring for each male, by combining all replicates, the mutation frequency of nitrosoguanidine was compared to that of the control by Fisher's exact test for each of the four broods separately and for the combined broods. All statistical tests were conducted at the 0.05 level of significance.

Deviations from SOP/Protocol

No deviations from the Standard Operating Procedures and GLP Protocol 86001 were made during this study.

Use of dimethyl sulfoxide (DMSO) as a solvent was avoided as recommended by the EPA (13).

RESULTS

The frequencies of spontaneous mutation for nitrosoguanidine and the negative control were 0.049% and 0.050% based on 8204 and 8076 X-chromosomes, respectively. The mutation frequencies for the positive control, 1-mM ethylmethane sulfonate, was 15.008%. The mutation frequencies for each compound are presented in Table 1. The mutation frequencies for each brood for nitrosoguanidine and the negative control are presented in Table 2. No significant difference was detected between the mutation frequency of the negative control and the nitrosoguanidine with the Fisher's exact test ($p = 1.000$). Also, no significant differences were detected between the negative control and the nitrosoguanidine for the data of broods 1, 2, 3, and 4 (Table 2).

Table 1

PERCENT MUTATION FREQUENCIES IN THE SEX-LINKED RECESSIVE LETHAL TEST OF NITROSOGUANIDINE*

Compound	1	2	3	4	Total Mutations	(Percent) Mutations
Nitrosoguanidine	1/1299	1/2077	0/2406	2/2422	4/8204	(0.049)
Negative Control	0/1470	1/1758	1/2422	2/2426	4/8076	(0.050)
Positive Control	37/183	58/235	51/419	44/429	190/1266	(15.008)

*Data are recorded as number of SLRL events/number of X-chromosomes tested.

Nitrosoguanidine: 25 male *Drosophila melanogaster* flies (CS strain) formed the P generation.

Negative Control: 25 male *Drosophila melanogaster* flies (CS strain) formed the P generation.

Positive Control: 5 male *Drosophila melanogaster* flies formed the P generation.

Table 2

FISHER'S EXACT TEST FOR SIGNIFICANCE OF THE DIFFERENCE BETWEEN NITROSOGUANIDINE AND NEGATIVE CONTROL IN SEX-LINKED RECESSIVE LETHAL TEST

	Brood Number			
Compound	1	2	3	4
Nitrosoguanidine	3/2307	0/2072	1/1999	0/1826
Negative Control	3/2392	1/2052	0/1908	0/1724
Positive Controls	70/376	56/374	46/291	18/225
p values	1.0000	0.4977	1.0000	--

Nitrosoguanidine: Nitrosoguanidine was suspended in a 1% carboxymethyl-cellulose and 1% fructose solution in deionized H_2O. Data are from 25 male *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc strain female flies each.

Negative Control: 1% carboxymethyl-cellulose and 1% fructose in deionized water. Data are from 25 *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

Positive Control: 1.0 mM ethylmethane sulfonate in 1% carboxymethyl-cellulose and 1% fructose in deionized H_2O. Data are from 5 male *Drosophila melanogaster* flies (CS strain) x 4 replicates mated with 3 Basc females each.

DISCUSSION

Recessive lethals are widely considered to be one of the most sensitive indicator of genetic alteration in *Drosophila melanogaster*. The results of this study indicate that nitrosoguanidine is not mutagenic when evaluated in the SLRL test in *in vivo* model for point mutations. These findings also confirm previous studies from this laboratory that indicated nitrosoguanidine was not mutagenic when evaluated in the Ames *Salmonella*/Mammalian Microsome Assay (14).

CONCLUSION

The results of this study indicate that nitrosoguanidine is not mutagenic when evaluated in the *Drosophila melanogaster* sex-linked recessive lethal test.

REFERENCES

1. Kaplan DL, Cornell JH, Kaplan AM. Decomposition of nitroguanidine. Environ Sci Technol, 1982; 59:488-492.

2. Kenyon KF. A data base assessment of environmental fate aspects of nitrosoguanidine. Frederick, MD: U.S. Army Bioengineering Research and Development Laboratory; 1982, DTIC No. ADA 125591.

3. Vogel E, Sobels FH. The function of *Drosophila* in genetic toxicology testing. In: Chemical Mutagens: Principles and Methods for Their Detection, edited by A. Hollaender, Vol. 4. New York: Plenum Press, 1976:93-141.

4. Abrahamson S, Lewis EB. The detection of mutations in *Drosophila melanogaster*. In: Chemical Mutagens: Principles and Methods for Their Detection, edited by A. Hollaender, Vol. 2. New York: Plenum Press, 1976:461-487.

5. Anonymous. Cancer testing technology and saccharin. Washington, D.C.: Office of Technology Assessment. October 1977.

6. Lee WR, Abrahamson S, Valencia ES, Von Halle FE, Wurgler FE, Zimmerling S. The sex-linked recessive lethal test for mutagenesis in *Drosophila melanogaster*. Mutat Res 1983; 123:183-279.

7. Wurgler FE, Sobels FH, Vogel E. *Drosophila* as assay system for detecting genetic changes. In: Handbook of Mutagenicity Test Procedures, edited by B. J. Kilbey et al. New York: Elsevier Scientific Publishing Co., 1977:335-373.

8. Brusick D. Principles of genetic toxicology. New York: Plenum Press, 1980:262-266.

9. Spencer WP, Stern C. Experiments to test the validity of the linear r-dose/mutation frequency relation in *Drosophila* at low dosage. Genetics 1948; 33:43-74.

10. Graf V. Spontaneous mutations in *Drosophila melanogaster*. Humangenetik 1972; 16:27-32.

REFERENCES (Contd.)

11. Alderson T. Chemically-induced delayed germinal mutation in *Drosophila*. Nature 1965; 207:164-167.

12. Dixon WJ, Brown MB, Engelman L, Frane JW, Hill MA, Jennrich RI, Toporek JD. BMDP statistical software. Berkeley: University of California Press, 1983:153,154,205.

13. Anonymous. Sex Linked Recessive Lethal Test in *Drosophila melanogaster*. HG-Gene Muta-Insects, Washington, D.C.: Office of the Toxic Substances, Environmental Protection Agency, August 1982.

14. Sebastian SE, Korte DW. Mutagenic Potential of Nitrosoguanidine in the Ames Salmonella/Mammalian Microsome Mutagenicity Test. Letterman Army Institute of Research, Presidio of San Francisco, California, 1988: Institute Report No. 259.

APPENDICES

Appendix A: CHEMICAL DATA

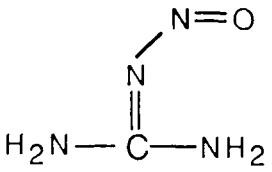
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: [illegible]

Lot Number: WCC-2-002

LAIR Code: TP48

Chemical Structure:



Molecular Formula: CH_4N_4O

Molecular Weight: 88

Physical State: Yellow powder

Analytical Data:

Nitrosoguanidine was analyzed by HPLC using conditions similar to those employed by Burrows et al.[1] Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flow-rate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.[2] IR (KBr) 3378, 3096, 1690, 1649, 1508, 1341, 1266, 1134, 1088, 1035, 690, 668 cm^{-1}.[3]

---

[1]Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and s-triazines in water. J Chromatogr 1984;16:494-8.

[2]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]*Ibid.* p 30.

Appendix A (Contd.): CHEMICAL DATA

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 l/moles·cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.76 mg/ml.[4]

Stability:

Stable for at least 4 hours in aqueous solution (pH 7.3) at 37°C.[5]

Source: Alan Rosencrance
U.S. Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Maryland

---

[4]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[5]Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 46. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B. HISTORICAL LISTING OF SIGNIFICANT EVENTS

Date	Event
3 June 1986 - 6 June 1986	Begin Replicate 1 (Run 62).
2 Dec 1986 - 5 Dec 1987	Begin Replicate 2 (Run 63).
16 March 1987 - 19 March 1987	Begin Replicate 3 (Run 64).
8 June 1987 - 11 June 1987	Begin Replicate 4 (Run 65).

Distribution List

Commander
US Army Biomedical Research and
Development Laboratory **(27)**
ATTN: SGRD-UBZ-C
Fort Detrick, Frederick, MD 21701-5010

Defense Technical Information Center
(DTIC) **(2)**
ATTN: DTIC-DLA
Cameron Station
Alexandria, VA 22304-6145

US Army Medical Research and
Development Command **(2)**
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, MD 21701-5012

Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234

Chief
USAEHA Regional Division, West
Fitzsimmons AMC
Aurora, CO 80045

Chief
USAEHA Regional Division, North
Fort George G. Meade, MD 20755

Chief
USAEHA Regional Division, South
Bldg. 180
Fort McPherson, GA 30330

Commander
USA Health Services Command
ATTN: HSPA-P
Fort Sam Houston, TX 78234

Commandant
Academy of Health Sciences
United States Army
ATTN: Chief, Environmental
Quality Branch
Preventive Medicine Division
(HSHA-IPM)
Fort Sam Houston, TX 78234

Commander US Army Materiel
Command
ATTN: AMSCG
5001 Eisenhower Avenue
Alexandria, VA 22333

Commander
US Army Environmental Hygiene
Agency
ATTN: Librarian, HSDH-AD-L
Aberdeen Proving Ground, MD 21010

Dean
School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20014

Commander
US Army Materiel Command
ATTN: AMCEN-A
5001 Eisenhower Avenue
Alexandria, VA 22333

HQDA
ATTN: DASG-PSP-E
Falls Church, VA 22041-3258

HQDA
ATTN: DAEN-RDM
20 Massachusetts, NW
Washington, D.C. 20314

6/88